Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Herd-prevalence of *Coxiella burnetii* (Q fever) antibodies in dairy cattle farms based on bulk tank milk analysis

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ARTICLE INFO

Article history:
Received 11 November 2010
Received in revised form 27 November 2010
Accepted 15 December 2010
Available online 20 January 2011

Keywords:
Q fever
Coxiella burnetii
Bulk tank milk
Dairy cattle
ELISA
Iran

ABSTRACT

Objective: To determine the prevalence of *Coxiella burnetii (C. burnetii)* antibody positive randomly selected dairy herds in southeast Iran (Kerman). **Methods:** Bulk tank milk samples were collected randomly from 44 sufficiently large commercial dairy herds, included near 12 000 dairy cattle, in Kerman (The largest province of Iran), southeast Iran. The samples were tested for antibodies against *C. burnetii* using the commercial CHEKIT® Q fever antibody ELISA Test Kit (Idexx, Liebefeld-Bern, Switzerland). **Results:** The prevalence of positive, negative and intermediate herds were 45.4%, 43.2% and 11.4%, respectively. **Conclusions:** The result supports the hypothesis of high prevalence and endemic pattern of Q fever in Iran. This investigation highlights the importance of further studies on Q fever in Iran.

1. Introduction

Zoonoses or diseases transmitted from animals to man, have been recognised as important public health issues for centuries and much of the early history of veterinary science was focused on the control of diseases such as bovine tuberculosis. Ungulates, in particular, are known to carry at least 315 zoonotic pathogens and many emerging and re-emerging infectious disease problems globally are zoonotic[1,2]. Q fever is a highly contagious zoonotic disease caused by the intracellular pathogen *Coxiella burnetii (C. burnetii.)* Multiple hosts can serve as a reservoir of infection. However, cattle, sheep and goats are the major reservoirs of *C. burnetii*[3]. Infected female animals shed a high concentration of the organism into birth products and smaller concentrations in urine, feces and milk. This shedding may continue over several months, particularly in vaginal mucus, feces and milk, even in those females with normal parturition[4,5]. All domesticated ruminants are susceptible but, with the exception of reproductive failures such as abortions, stillbirths, infertility and weak offspring, animals are usually asymptomatic and can remain chronically infected[6]. Q fever is an occupational hazard for veterinarians, abattoir workers, dairy farmers, and anyone with regular contact with livestock or their products. After primary infection, about 60% of humans are asymptomatic while 40% manifest clinical signs consisting of isolated fever, hepatitis and pneumonia. Endocarditis is the major clinical presentation of chronic Q fever[3]. There is very little detailed epidemiological data regarding the distribution and determinants of Q fever infection in cattle from anywhere in the world. The organism is present in cattle populations from all regions of the world with the notable exception of New Zealand. In Italy, there has been a clear suggestion that the seroprevalence is higher in housed cattle than cattle kept outdoors. The seroprevalence rates reported in cattle populations vary greatly, ranging from 3.4% to 84%[7]. In dairy herds, the level of antibodies to bovine viral diarrhoea virus, *Brucella melitensis* or *Neospora caninum* in bulk tank milk (BTM), measured by ELISA, has been reported as an inexpensive and valuable tool to assess the within-herd prevalence of seropositive cows in a herd[8-

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10]. ELISA tests are currently applied to BTM to identify and monitor herds in the control and eradication programmes for BVDV[11- 13]. The aim of the present study was to determine the prevalence of *C. burnetii* antibody positive randomly selected dairy herds in southeast Iran (Kerman).

2. Materials and methods

2.1. Population study and milk sampling

BTM samples were collected randomly from 44 sufficiently large commercial dairy herds, included near 12 000 dairy cattle, in Kerman (The largest province of Iran), southeast Iran. Samples were collected from late January to early April 2010. At the same time of BTM sampling, epidemiological information of these farms was obtained from the farmers and veterinary practitioner's in-charge. 50 mL of BTM per farm were collected into sterile plastic tubes, and sent to the microbiology laboratory of the Veterinary Medicine Faculty of Shahid Bahonar University of Kerman in a cool box on ice, for processing by ELISA.

2.2. ELISA analysis

Upon arrival, the samples were centrifuged, the fat fraction was removed and discarded, and the non-fat fraction was frozen to be tested for antibodies at a later time. The samples were tested for antibodies against *C. burnetii* using the commercial CHEKIT® Q fever antibody ELISA Test Kit (Idexx, Liebefeld-Bern, Switzerland). The ELISA plates were coated with *C. burnetii* inactivated phase 1 and phase 2 antigens. The optical densities (OD) of the samples were corrected by subtracting the OD of the negative control. The remaining non-fat fraction of the milk samples was frozen and stored for possible later purposes like determination of *C. burnetii*. The results were expressed as S/P values and estimated as the ratio between OD of the sample (S) and the OD of positive control (P) included in the test kit. According to the instructions from IDEXX S/P$\geqslant$40% was considered positive, S/P<30% was considered negative, and results in the interval 30%$\leqslant$S/P<40% were considered to be intermediate. The laboratory results were entered into SAS for estimating the prevalence overall.

3. Results

The prevalence of positive, negative and intermediate herds were 45.4%, 43.2% and 11.4%, respectively (Figure 1). Analysis of the BTM samples demonstrated S/P values ranging from 0 to 198 (Figure 2).

4. Discussion

Very few studies have been conducted on Q fever in Iran and this survey is the first study in dairy cattle farms based on BTM analysis by ELISA in Iran. The present study included 44 simple random selected industrial dairy herds in Kerman and was sufficiently large to estimate the prevalence of *C. burnetii* antibodies. According to the results, twenty herds (45.4 %) were positive by ELISA in this study. The preliminary studies were conducted by authors indicate people and animals in Iran are exposed to *C. burnetii*[14-16]. *C. burnetii* was detected in 6.2% bovine milk samples in Iran by PCR[17].

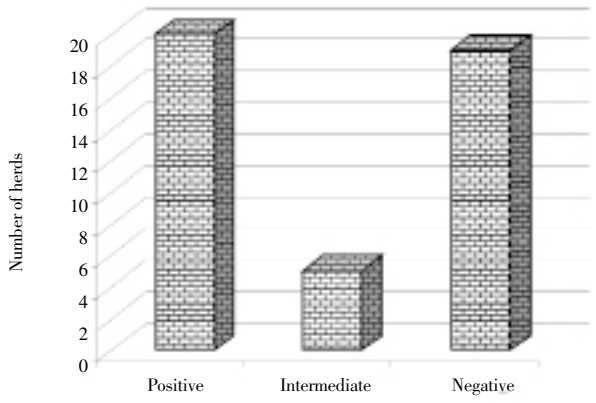

Figure 1. Number of positive, intermediate and negative random selected dairy herds of Kerman.

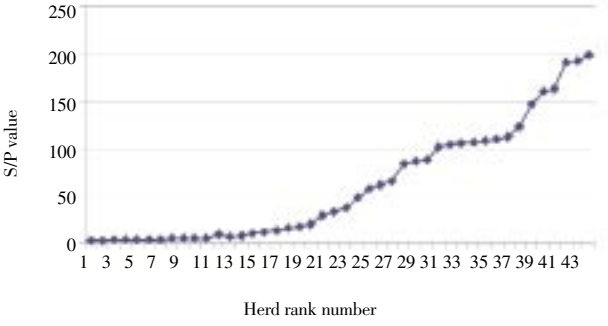

Figure 2. Array of antibody S/P values to *C. burnetti* in BTM samples from 44 random selected dairy herds of Kerman.

Agger *et al* demonstrated a prevalence of 59% antibody positive herds by same kit and methods in BTM samples in Danish dairy herds[18]. Results of this study confirm that antibody positive herds are very prevalent in this area and possibly other province of Iran. This high prevalence was surprised because in the pervious study we suggested 10.75% serpositivity in this area in dairy cattle herds[16]. The previous study was based on the CHEKIT Q-fever test kit, like in the present study. In the previous study we used individual sera samples from restricted farms (93 sera from 12 dairy cattle farms)[16] but in this study we used BTM samples from 44 dairy herds included near 12 000 dairy cattle.

The frequency estimates are apparent prevalence. This implies that the true prevalence may be different if the

test sensitivity and test specificity are less than 100%. However, the sensitivity and the specificity are unknown. Comparison of the test with the widely used complement fixation test (CF) showed good agreement in some studies and less good agreement in other studies. However, as the ELISA test is generally considered better than the CF test[18]. BTM sample is easy and inexpensive to collect, could be used to assess, on a larger scale at a low cost, the efficiency of control schemes aimed at controlling and/or preventing *Coxiella* shedding in dairy herds[19]. Q fever is apparently hyperendemic in Iraq and many US soldiers serving in this area have been exposed to *C. burnetii* and diagnosed as suffering by Q fever[20]. In common with all zoonotic diseases, control of the disease in animals will influence the level of disease seen in Man. BTM samples is good samples for screening of dairy herds for *C. burnetii* antibodies.

Vaccines can prevent abortion in animals, and it is evident that a *C. burnetii* phase I vaccine must be used to control the disease and to reduce environmental contamination and thus, the risk of transmission to humans. The widespread application of such a vaccine in cattle in Slovakia in the 1970s and 1980s significantly reduced the occurrence of Q fever in that country. Reducing exposure to raw milk for at risk people (pregnant women, patients with cardiac pathology or immunosupressed) and promoting the use of pasteurized milk and its products will also contribute to lowering the prevalence of Q fever[21].

Further improvements in the occupational hygiene of the work environment to prevent exposure to dusts containing *C. burnetii*, and an increased awareness of the presence of this microorganism in cattle, goat and sheep in our area, are necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This research was financially supported by Research Center for Tropical and Infectious Diseases, Kerman University of Medical Sciences, Kerman, Iran and Shahid Bahonar University of Kerman, Iran.

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